

Taxonomic Study of Marine Tardigrades from Korea III. A New Species of the Genus *Orzeliscus* (Heterotardigrada, Halechiniscidae)

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ABSTRACT

A new marine tardigrade species of the genus *Orzeliscus* belonging to the family Halechiniscidae is described from the sea coasts of Korea and Japan. This new species is most characterized in having slender, pole-shaped clava with uniform breadth along its whole length. Furthermore, it evidently differs from the congeners by the combination of characters of a hemispherical protrusion on cheek region of the head, a big and bulbous lateral projection between leg III and leg IV, and an elongate papillus terminating with a minute tube on leg IV. '*Orzeliscus* cf. *belopus*' *sensu* McKirdy, Schmidt and McGinty-Bayly, 1976 from the Galapagos Islands quite resembles this new species in sharing the slender, pole-shaped clava. However, these two Pacific populations are distinguished to each other by body size and shapes of the protrusion on cheek region and the lateral projection between leg III and leg IV. Scanning electron microscope photographs and a key to species of the genus *Orzeliscus* are also provided herein.

Keywords: Arthrotardigrada, East Asia, Japan, morphology, Northwest Pacific

INTRODUCTION

Taxonomic studies of marine tardigrades are very scarce in Korea. Only three articles have been provided for ten species of two genera in the families Batillipedidae and Halechiniscidae: eight *Batillipes* species of *B. longispinosus* Chang and Rho, 1997, *B. orientalis* Chang and Rho, 1997, *B. rotundiculus* Rho, Min and Chang, 1999, *B. pennaki* Marcus, 1946, *B. similis* Schulz, 1955, *B. tridentatus* Pollock, 1989, *B. crassipes* Tchesunov and Mokievsky, 1995, and *B. philippinensis* Chang and Rho, 1997 (see Chang and Rho, 1997; Rho et al., 1999); two *Halechiniscus* species of *H. jejuensis* Chang and Rho, 2002 and *H. remanei* Schulz, 1955 (see Chang and Rho, 2002). As the third of serial reports on the marine tardigrade fauna of Korea, a new species of the genus *Orzeliscus* is described with scanning electron microscope (SEM) photomicrographs and a key to species of the genus. Moreover, as a result of this study,

the Japanese specimens from southwestern coast of Honshu have turned out to be exactly same morphologically with the present Korean specimens, and are included in this paper as additional materials.

MATERIALS AND METHODS

Samplings were accomplished from intertidal to shallow sublittoral sandy bottoms. Upper 10 cm of sediments were scooped into polyethylene vinyl bags, and filtered through nylon net with a mesh size of 64 µm after freshwater rinsing for less than a minute for osmotic shock in the field. Samples were fixed in the field by immediately adding about 10% solution of formalin (equivalent of a 4% solution of formaldehyde in water by weight).

In the laboratory, tardigrades were sorted under a zoom-stereomicroscope (Leica M165C, Wetzlar, Germany) at 20–

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120× magnifications with a micropipette. Specimens were transferred to about 4% buffered formalin and mounted in glycerin on H-S slide (Shirayama et al., 1993) for double-coverglass preparation.

Drawings and measurements were made at 1,000× magnification using a drawing tube mounted on a differential interference contrast microscope with Nomarski optics (Leica DM2500).

Specimens for scanning electron microscopy were prepared as follows. Prefixation was done in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 17 hrs; after rinsing three times with 0.1 M phosphate buffer (10 mins each), we put them in 0.1 M phosphate buffer for 12 hrs. Postfixation was accomplished in 2% OsO₄ in 0.1 M phosphate buffer for 2 hrs; after rinsing three times with 0.1 M phosphate buffer (10 mins each), specimens were soaked in 0.1 M phosphate buffer for 12 hrs. Dehydration was made through an ethanol series of 50–100% by 10% increments (three times in 10 mins each); tardigrades were moved into t-butyl alcohol for 1 hr, and then freeze-dried with ES-2030 (Hitachi, Japan). The material was coated with platinum in ion sputter (E-1045, Hitachi), and examined on a Hitachi S-4300 SEM at 10 kV.

Holotype specimen is deposited in the National Institute of Biological Resources (NIBR), Incheon, Korea, and paratype specimens are kept temporarily in the collection of the first author (JL) at the Korea Institute of Ocean Science & Technology (KIOST), Korea. Additional materials from Korea and the voucher specimens from Japan are deposited in the Department of Biological Science, Daegu University (DB), Korea.

SYSTEMATIC ACCOUNTS

Order Arthrotardigrada Marcus, 1927

Family Halechiniscidae Thulin, 1928

Genus *Orzeliscus* du Bois-Reymond Marcus, 1952

Orzeliscus asiaticus sp. nov. (Figs. 1, 2)

Type locality. Baekripo Beach (36°48'44"N, 126°09'10"E; intertidal zone), Taean-gun County, Chungcheongnam-do Province, Republic of Korea.

Material examined. Holotype ♀ (NIBR00000779131), in glycerin on H-S slide, from the type locality, 20 Oct 2016 (leg. J Lee and AY Shin). Paratypes: 3 ♀♀ (KIOST01T01-01T03), mounted on H-S slides, collection details same as for the holotype. Nine specimens with the same collection data were mounted on an aluminium stub for scanning electronic microscopy.

Additional materials. *Korea:* Gangwon-do: 3 inds., Sokcho-si, Bongpo-ri, 2 Aug 1995, Lee J, Rho HS, Choi JW; 11 inds., Bongpo-ri, 11 Aug 1998, Rho HS, Choi JW; Gyeong-sangbuk-do: 1 ind., Uljin-gun, Jukbyeon-ri, 27 Sep 1997, Rho HS; 2 inds., Guryongpo-eup, Samjeong-ri, 29 Apr 1997, Chang CY, Rho HS; 5 inds., Guryongpo-eup, Samjeong-ri, 3 Jul 1997, Rho HS, Choi JW; Jeollanam-do: 1 ind., Wandogun, Isl. Shinjido, 8 Feb 2003, Chang CY, Lee J; Jeju-do: 1 ind., Jeju-si, Jocheon-eup, Hamdeok-ri, 21 Apr 1995, Chang CY, Lee J, Rho HS; 3 inds., Segwipo-si, Bomok-dong, Isl. Supseom, 21 Apr 1995, Rho HS, Choi JW; 4 inds., Isl. Supseom, 8 Nov 1997, Chang CY, Lee J, Rho HS; 2 inds., Isl. Supseom, 12 Oct 2002, Chang CY, Lee J. *Japan:* 1 ind. (DB40014, in glycerin on H-S slide), Shirahama Beach, 13 Oct 1996, Chang CY; 2 inds. (DB40015, in glycerin on H-S slide), Shirahama, Isl. Engetsudo, 14 Jan 1997, Chang CY, Rho HS; 3 inds. (DB40016, in ethyl alcohol), Tanabe Bay, Isl. Hatakejima, 18 Mar 1998, Chang CY, Lee J.

Diagnosis. Clava slender, pole-shaped, with nearly uniform breadth along its whole length; cheek region of head swollen, forming large hemispherical protrusion; lateral projection between leg III and leg IV big and bulbous, pointing posteriorly.

Description. Female (holotype): Body (Figs. 1A, B, 2A) very stumpy, 206.6 µm long from anterior end of head (excluding cephalic cirri) to posterior margin of trunk; broadest (94.5 µm) at level of lateral projections between leg III and leg IV; length to width ratio 2.2. Body not apparently divided with metameric constrictions. Dorsal cuticle strongly granulated, each granule rosette-shaped with fine wrinkles around centering nipple-like projection under scanning electron microscopy (Fig. 2G). Cheek region of head prominently swollen laterally, forming large hemispherical protrusion. Lateral body expansions not conspicuous, gently protruding laterally, except for lateral projection between leg III and leg IV, remarkably big and bulbous with its distal end pointing posteriorly (Figs. 1A, B, 2B, D). Posterior margin of body rounded, and convex mesially.

All cephalic cirri (Figs. 1A, C, 2A, C) organized with same cirrophorus-pattern: well-developed scapus basally, followed by flagellum proximally sheathed, then ended with hairy sensillum distally. Dorsal median cirrus relatively shorter than other cephalic cirri, 19.5 µm long, comprising scapus (5.6 µm) and flagellum (13.9 µm) including proximally sheathed portion (7.8 µm). Paired internal medial cirri issuing from ventral surface near rostral margin, 30.2 µm long, consisting of scapus (8.8 µm) and flagellum (21.4 µm, including 14.3 µm sheathed). Paired external medial cirri positioned between rostral margin and buccal aperture, slightly shorter than internal medial cirri, but seen as much shorter in dorsal view, 23.1 µm long (scapus 8.8 µm, flagel-

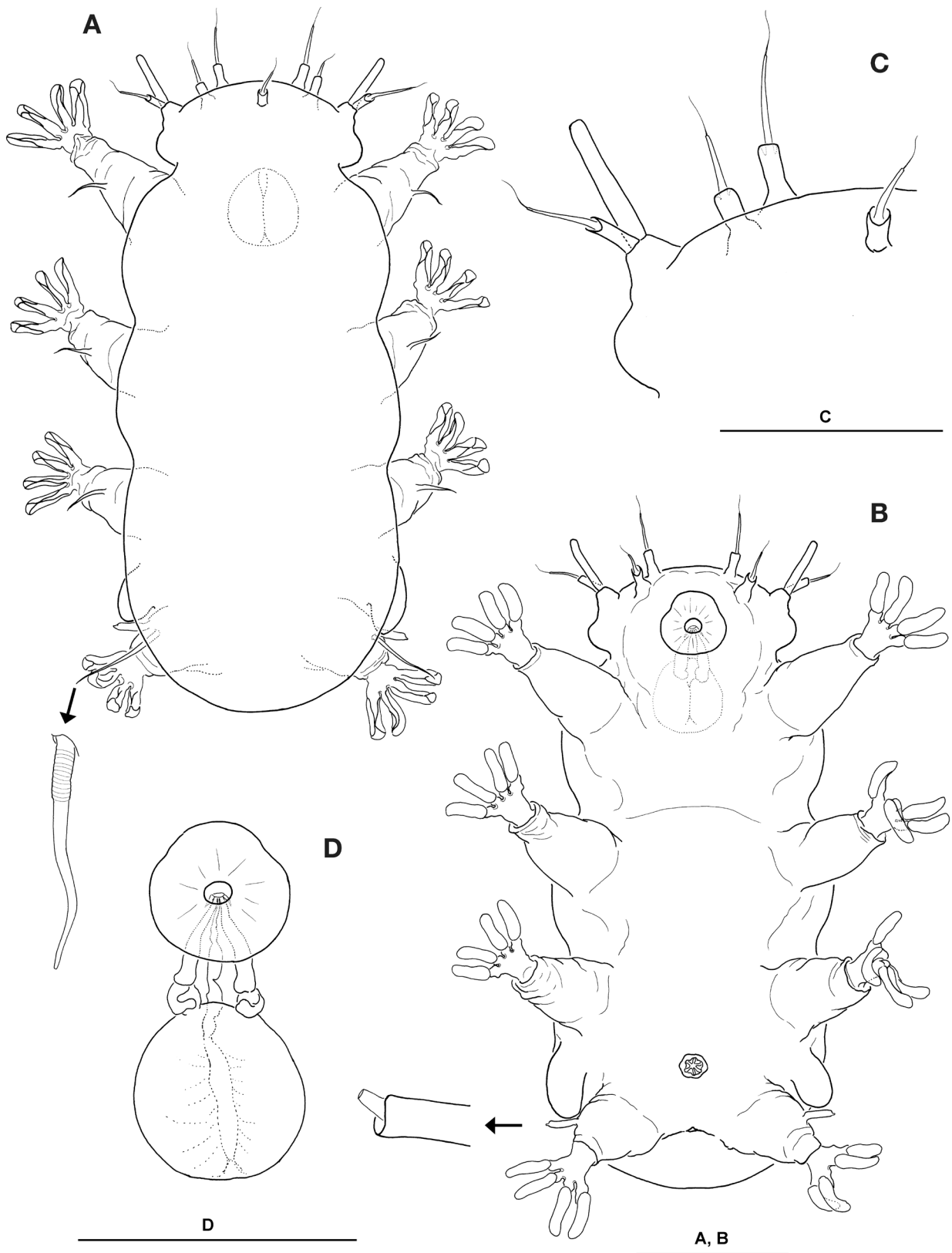


Fig. 1. *Orzeliscus asiaticus* sp. nov., female. A, Habitus, dorsal; B, Habitus, ventral; C, Cephalic appendages; D, Buccal apparatus. Scale bars: A, B, D = 50 μ m, C = 30 μ m.

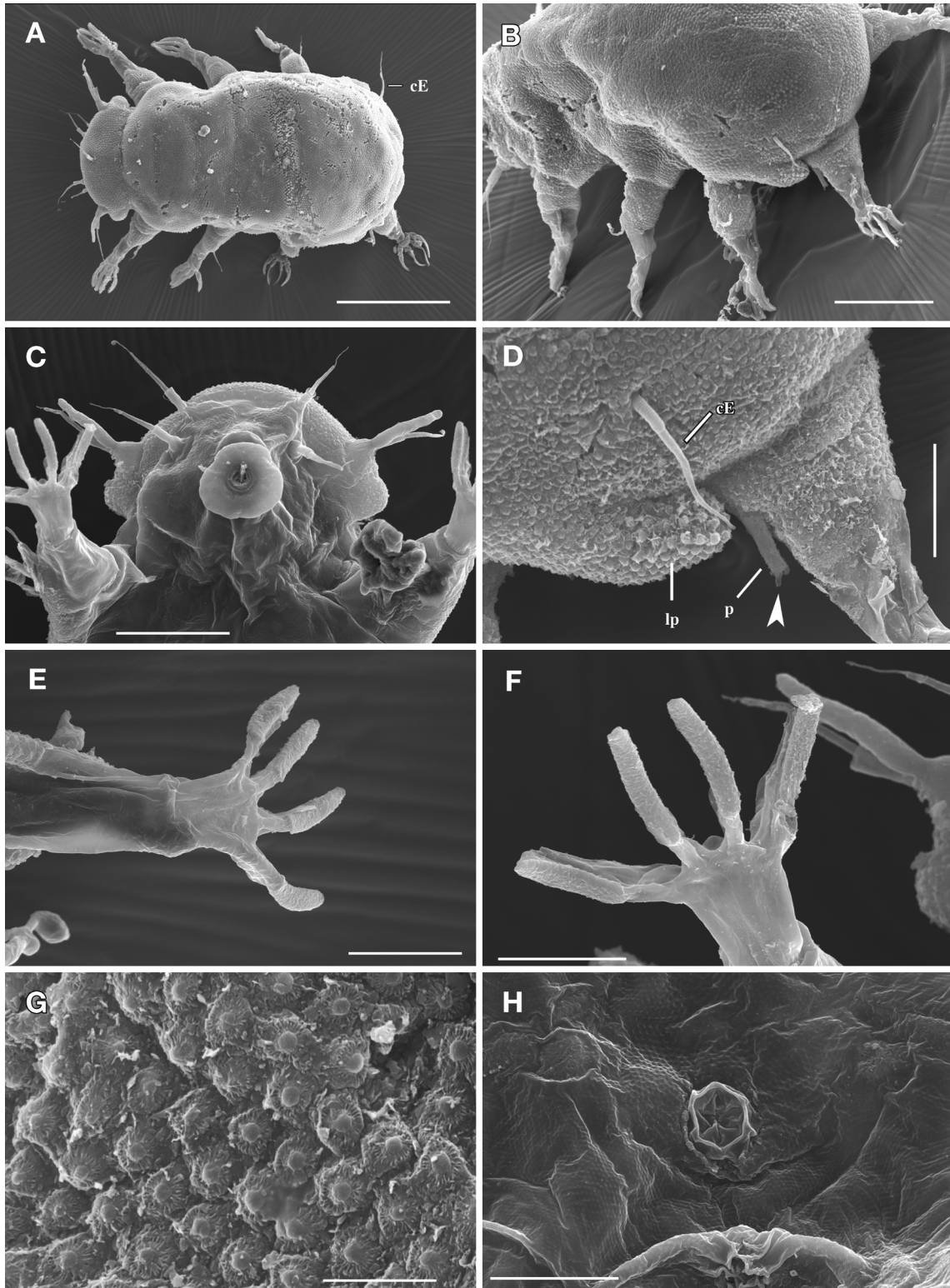


Fig. 2. *Orzeliscus asiaticus* sp. nov., female. (scanning electron microscope photographs). A, Habitus, dorsal; B, Left and rear sides of trunk and legs, dorsolateral view; C, Head and leg I (part), ventral; D, Lateral projection between leg III and leg IV, and a papillus on leg IV (arrowhead indicating a minute tubule terminating with a pore at distal end of spindle-shaped papillus); E, Leg I, ventral; F, Digits of leg I, ventral; G, Dorsal cuticle at the anterior part of body; H, Gonopore and anus, ventral. cE, cirrus E; lp, lateral projection; p, papillus. Scale bars: A=50 μ m, B=30 μ m, C, D=20 μ m, E, F, H=10 μ m, G=2.5 μ m.

lum 14.3 μm including 9.9 μm sheathed). Lateral sides of head strongly produced, each bearing lateral cirrus (cirrus A) dorsally and clava ventrally. Lateral cirri 22.6 μm long, consisting of 7.7 μm scapus and 14.9 μm flagellum (9.9 μm sheathed). Clava rather slender, pole-shaped, with nearly uniform diameter along all its length, 18.7 μm long, slightly shorter than lateral cirri (scapus of lateral cirrus not reaching to middle of clava).

Eye spot not observed. Buccal opening surrounded by round plate; anterior part of round plate smaller than posterior part (Figs. 1B, D, 2C), slightly wider than long (26.3 $\mu\text{m} \times 25.8 \mu\text{m}$). Pharyngeal apparatus (Fig. 1D) consisting of buccal tube (25.3 μm long), 2 crooked stylets (19.4 μm long) with well-developed stylet supports, and spherical pharyngeal bulb (32.4 μm long, 31.6 μm wide). Inner contents of pharyngeal bulb, such as pharyngeal bars, opaque and unclear, as surrounded by thick muscular material, especially in specimens fixed with formalin.

Legs (Figs. 1A, B, 2E, F) long, consisting of stout basal portion dorsally covered with granulate cuticle, a little narrowing mid-portion, and palm-like distal portion terminating with 4 digits. Digit forming long, blade-shaped plate, armed with mid-dorsal keel through its whole length; ventral surface seen as covered with thick, rough-hewn pad (see Fig. 2E, F). Legs I–III each furnished with 1 sensory bristle, positioned at distal part of dorsal cuticle, 13.3–14.4 μm long. Leg IV bearing elongate, spindle-shaped papillus terminating with minute tubule; papillus opened at its distal end, 12.2 μm long in total, issuing from anterior lateral surface of proximal part of leg (see Figs. 1A, B, 2B, D).

Cirrus E (Figs. 1A, 2A, B, D) 33.3 μm long, situated at posterior lateral corner of trunk, not simple and conical, but multi-articulate basally with repetitive ring-structures, as shown in Fig. 1A.

Female gonopore (Figs. 1B, 2H) situated ventrally at level between leg III and leg IV, relatively large, 7.8 μm in diameter, surrounded with 6 rosettes of small cuticular membrane. Distance between gonopore and anus scar 20.9 μm .

Male: Unknown.

Etymology. The proposed specific name *asiaticus* (Latin, meaning ‘Asian’) alludes to the localities, Korea and Japan.

Remarks. Two species are currently recognized in the genus *Orzeliscus*: *O. belopus* du Bois-Reymond Marcus, 1952 and *O. septentrionalis* Schulz, 1953 (see Guidetti and van der Land, 2016). The former is a semi-cosmopolitan species, and recorded from the Brazilian Atlantic coast (Du Bois-Reymond Marcus, 1952), the Northeast Atlantic coasts (Renaud-Debyser, 1963; Pollock, 1971; Renaud-Mornant and Goubault, 1980; Morgan and Lampard, 1986), New Caledonia Island (Renaud-Mornant, 1967), the Caribbean Sea (Pollock, 1982; Renaud-Mornant and Goubault, 1984), the Mediterranean

Sea (Grimaldi de Zio et al., 1983, 2003), and the Indian Ocean (Gallo et al., 2007). *Orzeliscus septentrionalis* was recorded from the North Sea (Schulz, 1953) and the Irish Sea (Moore, 1979), and in times past, regarded as a junior synonym of *O. belopus* (see Pollock, 1982) or treated as a *species inquirenda* (Guidetti and Bertolani, 2005). However, considering shape of the club-shaped clava, strong protrusion of the cheek region and absence of the papillary appendage on leg IV, *O. septentrionalis* should be recognized as a distinct valid species.

The present new species, *O. asiaticus* from Korea and Japan, is most characteristic in possessing slender, pole-shaped clava. Arrangement and shape of the cephalic appendages, especially morphological style of the clava has been dealt as a very significant character for classification of marine tardigrades as well as other interstitial groups such as thaumastodermatid gastrotrichs. The pole-shaped clava of the present new species is shown consistently in both Korean and Japanese specimens, and evidently differs from those of congeners, that is, apparently swollen at proximal portion in *O. belopus* (see Du Bois-Reymond Marcus, 1952; Morgan and King, 1976; Pollock, 1982; Ramazzotti and Maucci, 1983), while expanded toward distal portion and club-shaped in *O. septentrionalis* (see Schulz, 1953).

Another decisive character differentiating this new species from the congeners is a big lateral projection between leg III and leg IV. This lateral projection is either completely lacking in *O. belopus* (see Du Bois-Reymond Marcus, 1952; Pollock, 1982) and *O. septentrionalis* (see Schulz, 1953), or illustrated as a simple, weak, blister-like protrusion in a few records of *O. belopus* (see McKirdy et al., 1976; Morgan and King, 1976; Maucci, 1986). However, in the East Asian specimens, this lateral projection is consistently shown as a remarkably big and rather bulbous structure with its distal end pointing posteriorly.

Furthermore, *O. asiaticus* sp. nov. is distinguished from *O. belopus* by a hemispherical protrusion on cheek region, the apex of which extends far beyond the anterolateral corner of head, that is, the papillary projection bearing clava and lateral cirrus. *Orzeliscus septentrionalis* resembles this new species in having the hemispherical protrusion on cheek region.

‘*Orzeliscus* cf. *belopus*’ sensu McKirdy, Schmidt and McGinty-Bayly, 1976 from the Galapagos Islands (McKirdy et al., 1976) is different from the original description of Du Bois-Reymond Marcus (1952) as already mentioned by Ramazzotti and Maucci (1983), and supposedly a distinct valid species in considering the following discrepancies from *O. belopus* as well as *O. septentrionalis*: (1) rather small body, that is, less than 150 μm long (while about 200 μm long in the other two); (2) very elongate, pole-shaped clava, not swollen

len either proximally (as in *O. belopus*) or distally (as in *O. septentrionalis*); (3) the cheek region is quite weakly protruded, so the head is broadest at the lateral corner of forehead, against broadest at the cheek region in *O. septentrionalis*; and (4) a blister-like lateral protrusion between leg III and leg IV. '*Orzeliscus* cf. *belopus*' sensu McKirdy, Schmidt and McGinty-Bayly, 1976 resembles *O. asiaticus* sp. nov. in sharing the slender, pole-shaped clava. However, the two Pacific populations are clearly distinguished to each other by body size and shapes of protrusion on cheek region and lateral projection between leg III and leg IV. As for another old Pacific record of *O. belopus* from the New Caledonia by Renaud-Mornant (1967), its genuine identity cannot be confirmed here, because she did provide neither description nor figures for the two female specimens examined, except for very short comments only.

Orzeliscus asiaticus sp. nov. is one of the most frequent, though not most abundant, marine tardigrade species in Korea, which has occurred from moderately fine to coarse granite sands at the intertidal and sublittoral zone around seacoast of South Korea including Jeju Island, usually together with *Batillipes* species. This species was also collected repeatedly from beaches around the Seto Marine Biological Laboratory (SMBL) which locates at Tanabe Bay, southwestern coast of Honshu, Japan, when the authors visited the SMBL in summer and winter vacations during the period of 1996–2000. All the Japanese specimens examined completely coincide with Korean specimens morphologically throughout the significant characters above mentioned.

Key to species of the genus *Orzeliscus*

1. Clava club-shaped, swollen at distal portion; lacking the papillus on leg IV *O. septentrionalis*
- Clava not swollen distally; papillus present on leg IV ... 2
2. Clava much broader proximally, then abruptly narrowed at distal half; cheek region of the head not swollen strongly; lateral projection between leg III and leg IV lacking or very weak *O. belopus*
- Clava slender, pole-shaped, with nearly uniform breadth along its whole length; cheek region of head with hemispherical protrusion; lateral projection between leg III and leg IV big and bulbous *O. asiaticus* sp. nov.

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